

## Risk factors for *Campylobacter* spp. colonization in broiler flocks in Iceland

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### Abstract

We sampled 1091 Icelandic broiler flocks at slaughter from May 2001 to December 2003 to determine the prevalence of, and investigate risk factors for the presence of, *Campylobacter* spp. at the flock level. Approximately 15% of the flocks were positive for *Campylobacter* spp.; most (95%) of the infected flocks being raised during the months of April–September. Based on the data from the latter months, and using multivariable logistic regression with random effects for herd, we found that the odds of a flock being positive for *Campylobacter* spp. increased with age and flock size. Additionally, vertical ventilation systems were strongly associated with positive flocks (OR = 5.3). After controlling for these variables, we found no evidence of an effect of: year; company; *Campylobacter* being carried over from one flock to the next; time interval between flocks; using

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(at the hatcheries) eggs laid on the floor; density of bird housing, or the number of catch lots a flock was divided into for slaughtering purposes on the risk of a *Campylobacter*-positive flock.

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## 1. Introduction

*Campylobacter* spp. is an important agent of gastrointestinal disease in humans, heading the list of foodborne infections in many developed countries (Blaser, 1997; Allos, 2001; Frost, 2001). In a few cases, a more severe illness, the Guillain-Barré syndrome, has been associated with *Campylobacter* infection (Allos, 1997; Buzby et al., 1997; Hadden and Gregson, 2001).

Poultry meat is the main risk factor identified for human infection with *Campylobacter* spp. (Kapperud et al., 1992; Altekruse et al., 1999; Hudson et al., 1999; Allos, 2001), although other researchers point at different sources including milk and water (Hanninen et al., 2000; Corry and Atabay, 2001; Frost, 2001). Due to the difficulties associated with reducing the contamination of poultry carcasses at abattoirs, intervention measures at the farm level have been suggested as the most effective method to decrease *Campylobacter* prevalence in chicken meat (Herman et al., 2003). *Campylobacter* colonization in poultry is asymptomatic, suggesting that this bacterium is well adapted to the gut environment of the host. Commercially raised chickens rarely test positive for *Campylobacter* before 2 or 3 weeks of age, but they are capable of shedding large numbers of this organism in feces shortly after being colonized (Sahin et al., 2002; Newell and Fearnley, 2003). Once *Campylobacter* is introduced into a flock, it spreads quickly, and previous studies have found within-flock prevalences ranging between 60 and 100% (Gregory et al., 1997; Evans and Sayers, 2000; Heuer et al., 2001; Shreeve et al., 2002; Stern et al., 2003).

*Campylobacter* spp. colonization in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalences ranging from 18% to over 90%, with northern countries showing a lower proportion of positive flocks (Newell and Fearnley, 2003).

*Campylobacter* colonization in poultry usually follows a seasonal pattern, with a peak in the warmer months (Jacobs-Reitsma et al., 1994; Wedderkopp et al., 2000, 2001; Refregier-Petton et al., 2001; Bouwknecht et al., 2004). An exception to this finding has been reported in the United Kingdom, where several researchers found no seasonal effect (Humphrey et al., 1993; Evans and Sayers, 2000), although a seasonal variation in the concentration of *Campylobacter* in poultry has been described (Wallace et al., 1997). The reason behind this seasonal effect is largely unknown, although a possible role of migratory birds or insects has been suggested (Jacobs-Reitsma, 1997).

The possible sources and transmission routes of *Campylobacter* for poultry flocks have been investigated extensively, but no definitive factor(s) have been identified that explain the occurrence of the organism in commercial poultry flocks. Risk factors associated with horizontal transmission include lack of biosecurity measures (Humphrey et al., 1993; Jacobs-Reitsma et al., 1994; van de Giessen et al., 1996, 1998; Gibbens et al., 2001;

Herman et al., 2003; Cardinale et al., 2004), age (Berndtson et al., 1996b; Evans and Sayers, 2000; Bouwknecht et al., 2004), flock size (Berndtson et al., 1996b), carry-over from previous flock (Petersen and Wedderkopp, 2001; Wedderkopp et al., 2003), flock-thinning practices (Hald et al., 2000; Wedderkopp et al., 2000; Hald et al., 2001; Slader et al., 2002), contaminated air from adjacent poultry houses (Berndtson et al., 1996a), contaminated water (Kapperud et al., 1993; Pearson et al., 1993; Zimmer et al., 2003), other infected livestock on the farm (van de Giessen et al., 1996; Bouwknecht et al., 2004; Cardinale et al., 2004), mechanical transmission via insects (Berndtson et al., 1996a; Refregier-Petton et al., 2001), and infected wild birds (Chuma et al., 2000; Craven et al., 2000).

Some researchers have suggested that *Campylobacter* can spread from the parent flocks to the progeny (Pearson et al., 1996; Cox et al., 2002). However, most evidence suggests that vertical transmission plays a minor role, if any (Kazwala et al., 1990; van de Giessen et al., 1992; Pearson et al., 1993; Jacobs-Reitsma et al., 1995; Payne et al., 1999; Petersen and Wedderkopp, 2001; Sahin et al., 2003a).

In Iceland, domestic rates of human campylobacteriosis were highest in 1999, with 117.6 cases per 100,000, dropping to 32.7 per 100,000 in 2000. In poultry, the results of the Official Icelandic surveillance program reported a prevalence of *Campylobacter* in broiler flocks of 16% for 2000 (Reiersen et al., 2003).

To date, there has been no all-inclusive epidemiological study investigating what factors affect the prevalence of *Campylobacter* spp. in the Icelandic poultry industry. Due to the relative isolation of Iceland and the small size of both the poultry industry and the human population, plus the fact that Iceland does not import poultry meat or meat products, this country offers a unique opportunity to carry out a longitudinal study of *Campylobacter* at all production levels, from the breeder flocks to the consumer.

Our purpose was to estimate the prevalence of *Campylobacter* colonization at the flock level and to investigate the risk factors associated with the presence of *Campylobacter* spp. at slaughter in broiler flocks in Iceland. This study is part of a larger project investigating the transmission of, and risk factors for, *Campylobacter* at different levels of the poultry industry in Iceland, as well as for the human incidence of this disease (Stern et al., 2003, 2005).

## 2. Materials and methods

### 2.1. Data collection

#### 2.1.1. Epidemiological information

The target population in our study was the total number of commercial broiler flocks in Iceland slaughtered during 1 May–31 December, 2003. The study population consisted of all the broiler flocks slaughtered during that period and belonging to the three largest poultry companies in Iceland. Three remotely located farms that contributed approximately 10% of the broiler production in Iceland were excluded for practical reasons (Lowman, personal communication, 2004). Flock information was collected for the period May 2001–December 2003 by a field technician and the Veterinary Officer for Poultry Diseases, Icelandic Veterinary Services.

### 2.1.2. Bacteriological sampling and analysis

Broiler flocks were delivered to the slaughterhouses in 1–4 batches or ‘catch lots’, depending on flock size and other management factors. A catch lot was defined as a batch of birds collected from a flock on the same day for delivery to the slaughterhouse. From each catch lot, four pooled samples of 10 systematically selected ceca (including contents) were aseptically collected at the abattoir by the plant veterinarian. This sample size would ensure that *Campylobacter* spp. would be detected with 95% confidence if the within-flock prevalence was  $>7.2\%$  (Dohoo et al., 2003, p. 47). Previous studies from several countries, including Iceland, have shown that the prevalence of *Campylobacter* spp. in infected flocks is usually much higher than this (Gregory et al., 1997; Evans and Sayers, 2000; Heuer et al., 2001; Shreeve et al., 2002; Hein et al., 2003; Stern et al., 2003; Wedderkopp et al., 2003).

Following collection, samples were stored at 4 °C overnight and transported to the laboratory of the Institute for Experimental Pathology, University of Iceland, Keldur, Reykjavík. The microbiological procedures were as previously described; the epidemiologic sensitivity per fecal sample is not known, but presumed to be high because the analytic sensitivity is about 10 cfu/gm (Stern, personal communication; Stern et al., 2003, 2005). Briefly, serial dilutions of cecal contents were plated onto Campy-Cefex agar (Stern et al., 1992) and incubated at 42 °C for 48 h. Colonies with spiral-shaped cellular morphology, characteristic of *Campylobacter* spp., were further assessed using latex agglutination (Pan-Bio Inc., Baltimore, MD; Latex-Campy) as a serological confirmation test for *Campylobacter* spp. Although unknown, we assume a specificity approaching 100%.

### 2.2. Outcome variable

The study outcome was whether a flock was positive or not for *Campylobacter*. A flock was considered positive if *Campylobacter* spp. was isolated from at least one of the pooled samples from any of the catch lots at the slaughterhouse.

### 2.3. Risk factors

The 13 factors evaluated in our study are described in Tables 1 and 2. To specify the season when the flock was raised, we selected the month when the flock was 3 weeks old, because this is both the approximate mid point of age for broilers and the time after which most flocks are believed to become infected by *Campylobacter*, possibly due to depletion of maternal antibodies (Sahin et al., 2001, 2003b), although other factors might be involved. The date was determined by adding 21 days to the earliest hatching date for birds in the flock.

Farms that had both a vertical and a horizontal system were classified under vertical ventilation, because we believed that flocks would be at greater risk of colonization whenever vertical shafts, which provide easier access to wild birds and their feces, were present.

The variables ‘dryout period’ and ‘rest period between flocks’ were initially selected to investigate the ability of *Campylobacter* to survive in an empty house, and therefore were assessed using flocks that had a previous positive flock in the same house. These two

Table 1

Summary of potential risk factors available for analysis in a study of *Campylobacter* in Icelandic broiler flocks, May 2001–December 2003

Categorical variable	Description	Value	Number of flocks	% <i>Campylobacter</i> -positive
Season	Flock raised between April–September	Yes	586	95.0
		No	505	5.0
Year	Year the flock was raised	2001	210	17.6
		2002	411	17.3
		2003	470	12.8
Company	Company that owns the farm	1	370	17.3
		2	286	10.1
		3	435	17.2
Vertical ventilation	House had vertical ventilation shafts	Yes	918	17.4
		No	171	4.7
Use of floor eggs	Any supplying hatchery used eggs collected/laid on floor	Yes	205	18.1
		No	586	14.7
<i>Campylobacter</i> status of previous flock in same house	Positive	Yes	162	25.9
		No	826	14.3
Number of catch lots per flock	1–4 Lots	1	807	13.9
		2	231	16.9
		3	50	34.0
		4	3	0.0
Number of hatch lots that made up the flock	1–4 Lots	1	910	15.5
		2	124	17.7
		3	48	8.3
		4	9	11.1
Age at slaughter <35	Broilers up to 34 days old when delivered to slaughterhouse	Yes/no	101	5.9
Age at slaughter 35–36	Broilers 35 or 36 days old when delivered to slaughterhouse	Yes/no	319	14.4
Age at slaughter 37–38	Broilers 37 or 38 days old when delivered to slaughterhouse	Yes/No	275	16.0
Age at slaughter 39–41	Broilers 39–41 days old when delivered to slaughterhouse	Yes/no	303	17.8
Age at slaughter >41	Broilers 42 days old or older when delivered to slaughterhouse	Yes/no	60	25.0

Table 2

Summary of potential risk factors available for analysis in a study of *Campylobacter* in Icelandic broiler flocks, May 2001–December 2003

Continuous variables	Description	Flock <sup>a</sup>	p10	p50	p90
Size (number of broilers) <sup>b,c</sup>	Flock size	Positive	3745	6980	15436
		Negative	3123	6088	11873
Dryout period (days)	Date of disinfection to date of placement of next flock	Positive	3	8	23
		Negative	3	8	23
Rest period (days) <sup>b,c</sup>	Date of slaughter to date of placement of next flock	Positive	8	14	25
		Negative	7	15	31
Density <sup>b,c</sup>	Birds/square metre	Positive	13.4	18.4	23.2
		Negative	12.0	18.1	21.9

<sup>a</sup> *Campylobacter* status.

<sup>b</sup> Variable is significantly associated with *Campylobacter* status at  $p < 0.05$  in crude analysis.

<sup>c</sup> Variable is significantly associated with *Campylobacter* status at  $p < 0.05$  in crude analysis and after controlling “Farm”.

variables were also analyzed using all the flocks irrespective of the status of the previous flock, as surrogate indicators for other management practices. The analyses were carried out by considering these variables in both their continuous and categorical forms, using the quartiles as cut points.

## 2.4. Statistical analyses

For descriptive purposes we used an ordinary logistic regression model, ignoring the clustering by farm. Then, we screened each risk factor, using a “univariable” analysis based on a logistic model with random effects to control for clustering at the farm level (each farm had multiple flocks of birds during the study period). For the multivariable analysis we used a mixed-effects multiple logistic regression model of the form:

$$Y = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k + v_{\text{farm}} + \varepsilon$$

where  $\beta_0$  is the intercept,  $\beta_1 X_1 + \cdots + \beta_k X_k$  the part of the model representing the predictor variables,  $v_{\text{farm}}$  the random farm-effect component, controlling for farm as a clustering effect, and  $\varepsilon$  is the within-flock error term (Dohoo et al., 2003, p. 503).

Due to the low number of positive flocks in the winter period (9/506), and after initial analyses, it was decided to perform the multivariable risk factor analysis on flocks raised only in the April–September period. This decision was taken because some of the management variables such as ‘ventilation’ and cleaning could differ between the two periods. Additionally, when the whole dataset was initially used, we observed interactions between season and several other variables. These interactions were based on only a few

observations (due to the low number of positive flocks in winter), and consequently they had aberrant model parameters.

Provided that there was no collinearity ( $r < 0.8$ ) or strong association ( $OR < 8$ ) between variables, all variables with a Wald's  $p$ -value smaller than 0.25 in the "univariable" analysis were included in the multivariable model, and a backward elimination process was then carried out. Confounding was assessed every time a non-significant variable was dropped from the model by comparing the change in the coefficients for the variables remaining in the model. If the change was  $>25\%$ , confounding was considered to be present. Once the main-effects model was obtained, two-way interactions were generated and checked for significance. Models built by manual forward methods were used to help understand the major components of confounding.

Due to difficulties in assessing the goodness-of-fit of generalized linear models, we used the Hosmer–Lemeshow test in a fixed-effect model including farm as a categorical variable, with a significant ( $p < 0.05$ ) result indicating a poor fit. Additionally, Pearson and deviance residuals were computed to identify outlier observations and Cook's distance to identify possible influential observations (Dohoo et al., 2003, pp. 357–367). The impact of unusual observations was assessed by running the model without the observation, and comparing the coefficients between this model and the model using all the observations.

The statistical analyses were done using Stata Software (StataCorp. 2003; Stata Statistical Software: Release 8.0; College Station, TX, USA: StataCorp LP.).

### 3. Results

#### 3.1. Descriptive results

Data were available for 1091 flocks on 36 broiler farms. The median number of flocks per farm was 16.5 (minimum 2, maximum 193). The total number of flocks positive for *Campylobacter* spp. was 168 (15.4%; 95% CI: 13.3%; 17.7%), with only six farms (total 78 flocks, 58 of them in one farm) remaining negative for the duration of the study. Crude descriptive statistics of the flock-level factors are shown in Tables 1 and 2. Of the 56 positive flocks with more than one catch lot, 46 (82.2%) yielded *Campylobacter* spp. in samples collected from the first catch lot sent to the slaughterhouse. Six flocks were negative in the first lot but positive in the second, and four were negative for the first two catch lots and positive in the third. Of the positive flocks, 143 (85%) yielded *Campylobacter* spp. in all the samples collected.

There was a strong seasonal effect on the prevalence of *Campylobacter*, with 95% of the positive flocks being detected in the period April–September, peaking in July–August. This effect was present throughout the study period (Fig. 1). After controlling for season and farm, the annual flock prevalence of *Campylobacter* did not change significantly ( $p < 0.05$ ) during the study period.

The final number of flocks that were used in the statistical analysis of the flocks raised during the summer was 585, with a flock prevalence of *Campylobacter* spp. of 27%.

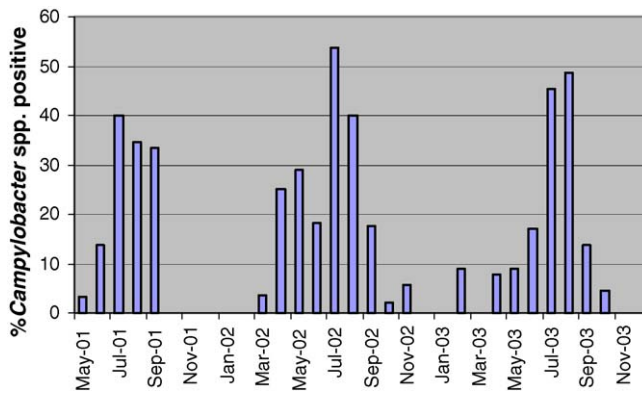


Fig. 1. Temporal distribution of *Campylobacter* spp. in Icelandic broiler flocks, May 2001–December 2003.

### 3.2. Univariable analysis (all flocks)

Only dryout period (whether previous *Campylobacter* status was considered or not) did not have  $p < 0.25$  in the logistic model controlling for farm (Table 1).

### 3.3. Multivariable analysis

Our final main-effect model for summer flocks included three significant ( $p < 0.05$ ) variables: ‘age at slaughter’ (as a categorical variable), ‘flock size’ and ‘vertical ventilation’ (Table 3).

Age was modeled as a categorical variable based on its relationship with the outcome being non-linear (using lowess smoothed plots), and its quadratic term being non-significant. Additionally, the different categories of ‘age at slaughter’ allowed for a simpler interpretation of the role of this variable. Compared to flocks slaughtered at ages  $<34$  days,

Table 3

Risk factors for *Campylobacter*-positive broiler flocks in Iceland, May 2001–December 2003 from a multivariable generalized linear model (logistic regression)

Variable	<i>b</i>	S.E. <sup>a</sup>	<i>p</i>	95% CI	OR
Flock Size/1000	0.08	0.02	$<0.001$	0.04, 0.11	
Increased by 5000 birds	–	–	–	–	1.47
Increased by 10000 birds	–	–	–	–	2.16
Vertical ventilation	1.66	0.66	0.012	0.37, 2.95	5.27
Age $<35$ days (Ref.)	0.00	Ref.	Ref.	Ref.	1.00
Age 35–36 days	1.06	0.58	0.067	–0.08, 2.19	2.87
Age 37–38 days	1.23	0.59	0.038	0.07, 2.40	3.43
Age 39–41 days	1.45	0.59	0.015	0.28, 2.61	4.26
Age $>41$ days	2.33	0.77	0.002	0.82, 3.84	10.3
Intercept	–4.23	0.82	$<0.001$	–5.84, –2.62	

<sup>a</sup> Robust standard errors allowing for clustering within Farm.



the odds of a flock to be *Campylobacter*-positive increased as the flock became older, as demonstrated by the increasing odds ratio (OR) (although not significant for flocks aged 35 or 36 days). 'Age at slaughter' data were not available for 29 flocks; however, the percent positive amongst these was 10%; essentially the same as the risk in those slaughtered at <35 days of age.

The relationship between 'flock size' and the probability of a flock being positive was visually assessed (again using lowess smoothed plots), and considered linear. In biological terms, an increase in flock size of 10,000 birds resulted in a two-fold increase in the odds of the flock being positive for *Campylobacter*, conditional on the other risk factors being kept fixed.

During the backward elimination process, it was noted that company (a non-significant ( $p > 0.05$ ) risk factor in the multivariable model) was a confounder for two other non-significant variables and one significant variable "flock size". 'Company' was strongly related to 'farm' and it also was highly confounded by 'flock size' and 'age at slaughter' (company 2 had the smallest average flock size and the youngest age at slaughter). Ultimately, we chose to drop 'company' because we viewed it as explanatory for the flock-size and age-at-slaughter variables and their effects provided a more useful basis for causal inferences.

Interaction terms were individually significant between 'vertical ventilation' and both 'flock size' and 'age at slaughter'. However, when both interaction terms were included in the model, the interaction with 'flock size' became non-significant, so it was eliminated from the model. The interaction between 'vertical ventilation' and 'age at slaughter' was subsequently found to be supported by one observation only – detected as an influential case – resulting in extreme OR values. Therefore this interaction term was also excluded from the model.

Several ( $n = 10$ ) outlier observations were identified, but it was decided to leave them in the model because there was no common pattern to them, and when removed, one at a time, they did not affect the coefficients in the model substantially. No influential cases were observed once the interaction term between 'vertical ventilation' and 'age at slaughter' was excluded from the model. The Hosmer–Lemeshow goodness-of-fit test indicated that the model fitted the data adequately ( $p = 0.552$ ). The mixed-effects model estimated an intra-class coefficient of 0.24 in the null model. The fairly low intra-class correlation coefficient (5.2%,  $p = 0.024$ ) for the clustering effect of farm in the final model suggested that most of the original farm-level clustering was explained by the three significant variables.

#### 4. Discussion

We found a relatively low flock prevalence of *Campylobacter* that was comparable to that of other northern European countries, with only Norway (Hofshagen and Kruse, 2003) and Finland (Perko-Makela et al., 2002) reporting lower rates. Iceland has an official policy of testing flocks prior to slaughter and requiring that meat derived from positive flocks be sold frozen. This policy results in a price penalty to the producers, who undoubtedly attempt to reduce or eliminate *Campylobacter* from their flocks by enhancing farm-biosecurity measures (Stern et al., 2003), which might help explain the low prevalence found in this study. Although the sensitivity of our pooled cecal culture is unknown, based

on the fact that almost all positive flocks were positive in all samples we believe the sampling and culture strategy has a very high (>90%) sensitivity at the flock level.

The seasonal distribution of *Campylobacter* in Icelandic flocks was the largest effect found in the study, and is consistent with research in most other countries (Newell and Fearnley, 2003). Due to the decision to use only observations from flocks raised during summer in our analyses, the inferences made for the remaining risk factors are valid solely for the summer months in the context of the Icelandic broiler industry.

The effect of increasing flock size on the odds of a flock being positive has been previously reported (Berndtson et al., 1996b), although other studies failed to find this association (Bouwknegt et al., 2004; Cardinale et al., 2004). In our case, a substantial rise in risk was predicted only for large increases in size (e.g. 10,000 birds) relative to the average size (median = 6200) of a broiler flock in Iceland. This effect was independent of bird density, but could be due to bigger flocks offering more chances for introduction of *Campylobacter* because of increased personnel movements, or larger volume of water and air used (both potential carriers of the pathogen).

Vertical ventilation was strongly associated with positive flock status. Gibbens et al. (2001) found a similar effect in a biosecurity trial, suggesting that horizontal fans are easier to clean and disinfect. Another possibility would be that vertical fans act as sources of heat for wild birds, which would then gather on the roof of the broiler house. This might lead to droppings falling into the house through the fan, either directly if the fan is not fully bird-proof, or washed in by rain. Wild birds are carriers of *Campylobacter*, and they might be sources of this pathogen (Craven et al., 2000; Newell and Fearnley, 2003). Studies in other Nordic European countries have found prevalences of *Campylobacter* spp. in wild birds ranging from 5% to 36% (Kapperud and Rosef, 1983; Broman et al., 2002; Waldenström et al., 2002). If wild birds were responsible for introducing *Campylobacter* into the broiler houses, this might explain the strong seasonal distribution observed, because the activity of wild birds in winter is greatly reduced. The role of ventilation systems deserves further attention, with special emphasis on the integrity of air outlets to ascertain whether fecal contamination from wild birds could be introduced into the house environment.

Our results show a higher risk of *Campylobacter* colonization associated with increasing age at slaughter. This is in agreement with prior studies (Berndtson et al., 1996b; Bouwknegt et al., 2004). A longer duration in the broiler house could mean more chances for *Campylobacter* to be introduced from the house environment. Additional time before slaughter would also allow for cecal-colony concentrations to become detectable (Stern et al., 2001). Taking this association into account, a policy of slaughtering flocks at a younger age might lead to a reduction in the prevalence of *Campylobacter*.

In univariable analyses, the number of catch lots was directly related to prevalence of positive flocks. However, most flocks with multiple catch lots were positive in cecal samples taken from the first catch lot. Furthermore, when flock size was controlled, the number of catch lots became non-significant. Finally we are aware that, in Iceland, special hygienic measures are taken by the catching crews to prevent such introduction, and these crews are usually made up of workers from the source farm, and they do not move from farm to farm. Hence, although there is a possibility of within-farm spread, we conclude that catching crews did not play an important role in introducing *Campylobacter* to most of the positive flocks.

Contrary to what some previous surveys have shown (Petersen and Wedderkopp, 2001; Wedderkopp et al., 2003), but in agreement with numerous others (van de Giessen et al., 1992; Humphrey et al., 1993; Jacobs-Reitsma et al., 1995; Pearson et al., 1996; van de Giessen et al., 1996; Payne et al., 1999; Shreeve et al., 2002), the *Campylobacter* status of the previous flock was not a predictor of future flock status in our study. Because cleaning and disinfection practices might be more of a farm-level than a flock-level variable, specific practices at the poultry house or farm level should be investigated.

Our study failed to reveal a link between using eggs laid on the floor in the breeder pens and *Campylobacter*-positive flocks, although there was a high number of missing values for this variable (28.6% of flocks). Therefore this result should be interpreted with caution. Eggs collected from the floor are more likely to be contaminated with feces, and it has been suggested that *Campylobacter* might survive on the egg surface and hence be a source of infection for newly hatched chicks (Doyle, 1984). However, in agreement with our findings, recent studies indicate that transmission of *Campylobacter* in eggs is a rare event (Newell and Fearnley, 2003; Sahin et al., 2003a).

The fact that some farms remained negative for the duration of the study period, and that most flocks were negative, reinforces the idea that it is possible to raise *Campylobacter*-free broilers. Further research of risk factors at the flock, farm and house level would help identify parameters we might have overlooked in our study of flock level variables.

## 5. Conclusions

*Campylobacter* spp. was present in approximately 15% of all the 1091 broiler flocks that were included in our study, with 95% of those positive flocks raised between April and September. We identified increasing age and flock size as risk factors for colonization in the summer months. Vertical ventilation was strongly associated with positive farms, and might indicate that *Campylobacter* is introduced from the environment through roof shafts.

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